

## Additional file 1: Tables

Additional file 1: Table S1. gRNA gene-target sequences, primers for PCR genotyping and RT-qPCR probes used in this study

Item	Exon/Intron	Sequence (5'-3')	Amplicon length (bp)	Annealing temp (°C)	Ensemble gene ID
<b>gRNA target:</b> <i>shank3b</i>	ex2	GGGCGTGTTGTTGCCACGGCCGG			ENSDART00000145613
<b>PCR genotyping</b>	in1	ATATGGGTCTTGCTTGTATACAGGCGA	500	60	
	in2	GAATCAACAACCTCGGTCTGTACCAATCA		60.3	
<b>RT-qPCR</b>					
<i>shank3b</i>	ex2	CGGCCGTGGCAACAACAC	245	59	ENSDART00000145613
	ex3	TTAAGCACATCGGTCAGGCTTTGT		59	
<i>β-actin</i>	ex2	CGAGCTGTCTTCCCATCCA	102	60	ENSDART00000091805.5
	ex3	TCACCAACGTAGCTGTCTTTCTG		60	
<i>shank3a</i>	ex5/6	GGCACTTATTACGCTGCTGGATCTG	191	60	
	ex6/7	CATGACGGCAAGCCTGGTGAAT		60.7	
<b>RFP</b>		CATGAAGCTGTACATGGAGGGCAC	219	59.4	
		GACTGCTTAAAGAAGTCGGGGATG		59	

**Additional file 1: Table S2. SHANK family gene sequences used in this study**

Gene	NCBI reference protein number	Amino acid	Species
<i>SHANK3</i>	NP_277052.1	1731	Human
<i>shank3a</i>	XP_017207498.1	1933	Zebrafish
<i>shank3b</i>	XP_009298459.1	1643	Zebrafish
<i>SHANK1</i>	NP_057232.2	2161	Human
<i>shank1</i>	NP_036441.2	1849	Zebrafish
<i>SHANK2</i>	NP_036441	1849	Human
<i>shank2</i>	XP_021326187.1	1810	Zebrafish

**Additional file 1: Table S3. Protein homology analysis of zebrafish shank3a and shank3b and human SHANK3**

Gene	NCBI reference mRNA number	NCBI reference protein number	Amino acid	vs. human SHANK3 protein		
				Query cover (%)	Identity (%)	Accession (human SHANK3)
<i>shank3a</i>	XM_017352009.2	XP_017207498.1	1933	95	59	NP_277052.1
<i>shank3b</i>	XM_009300184.3	XP_009298459.1	1643	97	55	NP_277052.1

**Additional file 1: Table S4. Protein homology comparison between zebrafish shank3a and shank3b**

Gene	NCBI reference mRNA number	NCBI reference protein number	Amino acid	shank3a VS. shank3b	
				Query cover(%)	Identity (%)
<i>shank3a</i>	XM_017352009.2	XP_017207498.1	1933	95	59
<i>shank3b</i>	XM_009300184.3	XP_009298459.1	1643		

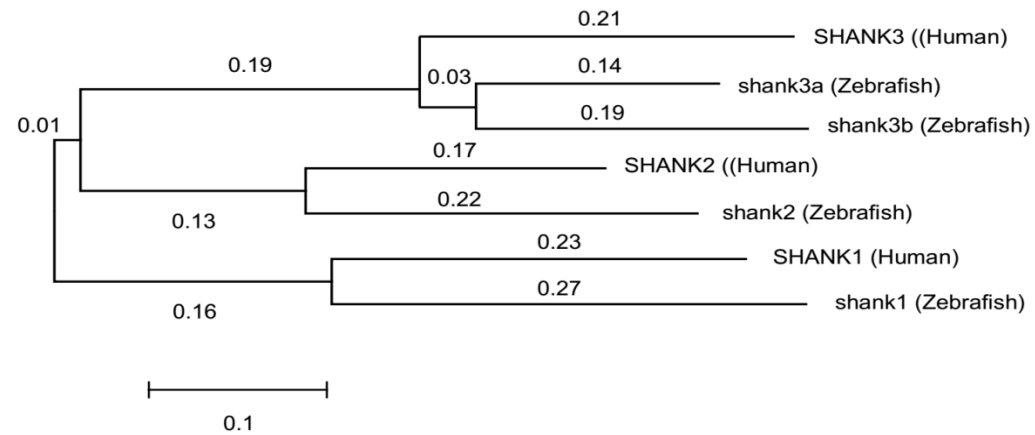
**Additional file 1: Table S5. Repetitive behaviors of *shank3b*<sup>-/-</sup> adult male zebrafish (3.5 mpf)**

	Figure "8"		Big circling		Small circling		Walling	
* No.	#shank3b <sup>+/+</sup>	shank3b <sup>-/-</sup>	shank3b <sup>+/+</sup>	shank3b <sup>-/-</sup>	shank3b <sup>+/+</sup>	shank3b <sup>-/-</sup>	shank3b <sup>+/+</sup>	shank3b <sup>-/-</sup>
1	0	15	0	2	0	2	4	2
2	2	1	0	8	0	0	3	4
3	0	1	0	6	0	0	0	0
4	0	2	0	6	1	2	2	2
5	0	14	0	5	0	1	4	8
6	0	8	1	1	0	2	2	0
7	1	6	1	0	2	2	2	0
8	0	8	0	2	0	7	0	2
9	1	2	0	4	0	0	0	8
10	0	4	0	0	0	0	3	3
11	2	18	2	2	0	1	0	2
12	0	2	0	0	1	0	0	7
13	0	1	1	3	1	0	0	3

\*, Tested adult (3.5 mpf) male zebrafish in this study.

#, Number of each type of repetitive behavior occurring in the examination window.

## Additional file 1: Figures



Additional file 1: Figure S1. Phylogenetic tree of evolutionary relationship of SHANK family proteins.

Sequences producing significant alignments:

Select: [All](#) [None](#) Selected:0

Alignments Download GenPept Graphics Distance tree of results Multiple alignment

Description	Max score	Total score	Query cover	E value	Ident	Accession
<input checked="" type="checkbox"/> <a href="#">SH3 and multiple ankyrin repeat domains protein 3 [Homo sapiens]</a>	1725	1725	95%	0.0	59%	<a href="#">NP_277052.1</a>
<input type="checkbox"/> <a href="#">SH3 and multiple ankyrin repeat domains protein 2 isoform 1 [Homo sapiens]</a>	1336	1336	95%	0.0	46%	<a href="#">NP_036441.2</a>
<input type="checkbox"/> <a href="#">PREDICTED: SH3 and multiple ankyrin repeat domains protein 2 isoform X2 [Homo sapiens]</a>	1334	1334	95%	0.0	46%	<a href="#">XP_016872878.1</a>
<input type="checkbox"/> <a href="#">PREDICTED: SH3 and multiple ankyrin repeat domains protein 1 isoform X3 [Homo sapiens]</a>	874	1044	53%	0.0	52%	<a href="#">XP_011525316.1</a>
<input type="checkbox"/> <a href="#">PREDICTED: SH3 and multiple ankyrin repeat domains protein 1 isoform X1 [Homo sapiens]</a>	870	1039	53%	0.0	51%	<a href="#">XP_011525315.1</a>
<input type="checkbox"/> <a href="#">SH3 and multiple ankyrin repeat domains protein 1 [Homo sapiens]</a>	868	1037	53%	0.0	52%	<a href="#">NP_057232.2</a>
<input type="checkbox"/> <a href="#">PREDICTED: SH3 and multiple ankyrin repeat domains protein 2 isoform X3 [Homo sapiens]</a>	755	755	77%	0.0	39%	<a href="#">XP_005277989.1</a>
<input type="checkbox"/> <a href="#">PREDICTED: SH3 and multiple ankyrin repeat domains protein 2 isoform X4 [Homo sapiens]</a>	609	609	65%	0.0	39%	<a href="#">XP_016872879.1</a>

Sequences producing significant alignments:

Select: [All](#) [None](#) Selected:0

Alignments Download GenPept Graphics Distance tree of results Multiple alignment

Description	Max score	Total score	Query cover	E value	Ident	Accession
<input checked="" type="checkbox"/> <a href="#">SH3 and multiple ankyrin repeat domains protein 3 [Homo sapiens]</a>	1400	1400	97%	0.0	55%	<a href="#">NP_277052.1</a>
<input type="checkbox"/> <a href="#">SH3 and multiple ankyrin repeat domains protein 2 isoform 1 [Homo sapiens]</a>	964	1103	92%	0.0	54%	<a href="#">NP_036441.2</a>
<input type="checkbox"/> <a href="#">PREDICTED: SH3 and multiple ankyrin repeat domains protein 2 isoform X2 [Homo sapiens]</a>	963	1102	92%	0.0	54%	<a href="#">XP_016872878.1</a>
<input type="checkbox"/> <a href="#">PREDICTED: SH3 and multiple ankyrin repeat domains protein 1 isoform X3 [Homo sapiens]</a>	875	1041	54%	0.0	54%	<a href="#">XP_011525316.1</a>
<input type="checkbox"/> <a href="#">PREDICTED: SH3 and multiple ankyrin repeat domains protein 1 isoform X1 [Homo sapiens]</a>	869	1034	54%	0.0	53%	<a href="#">XP_011525315.1</a>
<input type="checkbox"/> <a href="#">SH3 and multiple ankyrin repeat domains protein 1 [Homo sapiens]</a>	869	1034	54%	0.0	53%	<a href="#">NP_057232.2</a>
<input type="checkbox"/> <a href="#">PREDICTED: SH3 and multiple ankyrin repeat domains protein 2 isoform X3 [Homo sapiens]</a>	414	552	62%	8e-120	46%	<a href="#">XP_005277989.1</a>
<input type="checkbox"/> <a href="#">PREDICTED: SH3 and multiple ankyrin repeat domains protein 2 isoform X4 [Homo sapiens]</a>	287	426	51%	2e-78	47%	<a href="#">XP_016872879.1</a>
<input type="checkbox"/> <a href="#">SH3 and multiple ankyrin repeat domains protein 2 isoform 2 [Homo sapiens]</a>	237	375	42%	2e-62	54%	<a href="#">NP_573573.2</a>

**Additional file 1: Figure S2. Homology comparison of zebrafish shank3a and shank3b with human SHANK3**

**BLAST** ® >> blastp suite

Align Sequences Protein BLAST

---

blastn **blastp** blastx tblastn tblastx

Enter Query Sequence **shank3a** BLASTP programs search protein subjects using a protein query. [more...](#)

Enter accession number(s), gi(s), or FASTA sequence(s) Clear Query subrange

1681 rpyrlfgrs klwedvpr plsgaeghp twiselsarl qqlnkdtarl geeplgald  
 1741 pgrkspvpg rlfslgelh tigrsgytt ytirpgsryp vtrrtspgs pdrsdplgi  
 1801 rfglatapi tpttlkss lslphekev rfvrssar srspspasp gntsplltir  
 1861 pfhqpillw nkydygdla sinlsedr fqeheiegsh lpalkedyd elgvtvgrh  
 1821 mniaralkl les

From  To

Or, upload file 选择文件 未选择任何文件

Job Title

Enter a descriptive title for your BLAST search

☒ Align two or more sequences

---

Enter Subject Sequence **shank3b**

Enter accession number(s), gi(s), or FASTA sequence(s) Clear Subject subrange

1381 prylfgrsk lwgepragm gssdesrpa mgaelldkdt hslgeepag apldpgrsp  
 1441 vggarceeng gekslfsl gelhtisqr ygtftirpg srypvtrrt spgatperse  
 1501 plqprtfpg hhhhtlks szslpqpqk evrfvmsas ararsrsp spaspasp  
 1561 vlqapllur pfrqplaw skydygwle svlgehrur fleheiegah lpalkddla  
 1621 elgvtvgrh mniaralkl les

From  To

Or, upload file 选择文件 未选择任何文件

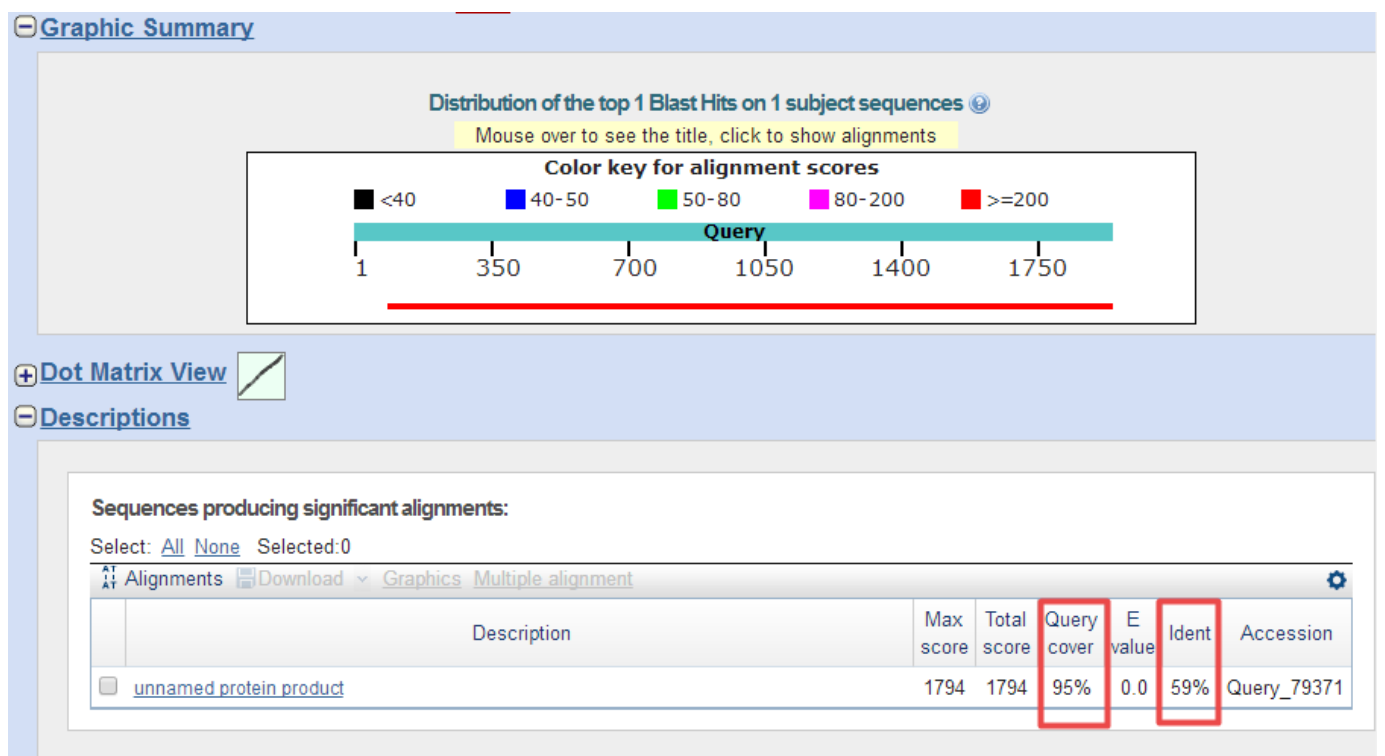
---

Program Selection

Algorithm ☒ blastp (protein-protein BLAST)  
 Choose a BLAST algorithm

---

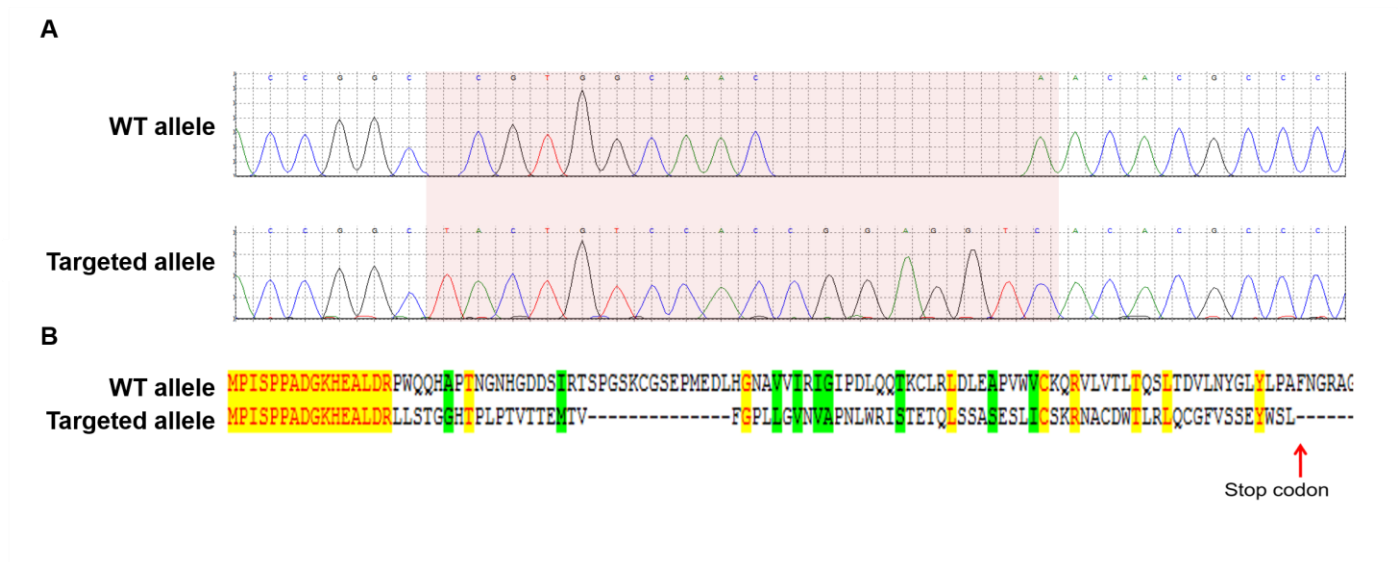
**BLAST** Search protein sequence using Blastp (protein-protein BLAST)  
☒ Show results in a new window



**Additional file 1: Figure S3. Homology comparison between zebrafish shank3a and shank3b.**

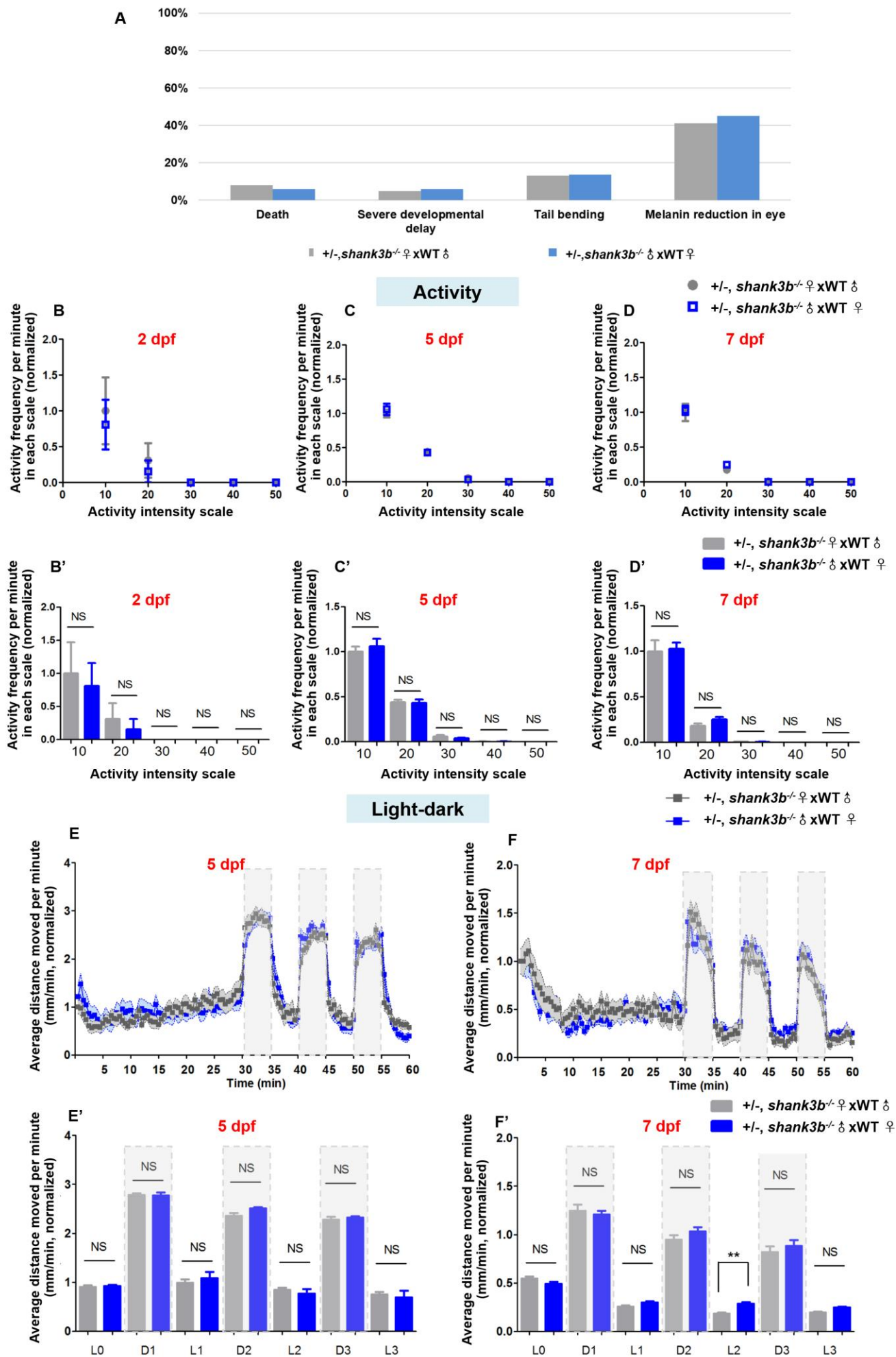
We used the “Protein BLAST” tool of NCBI website

([https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins&PROGRAM=blastp&BLAST\\_PROGRAMS](https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins&PROGRAM=blastp&BLAST_PROGRAMS)) to compare the homology of zebrafish shank3a and shank3b.

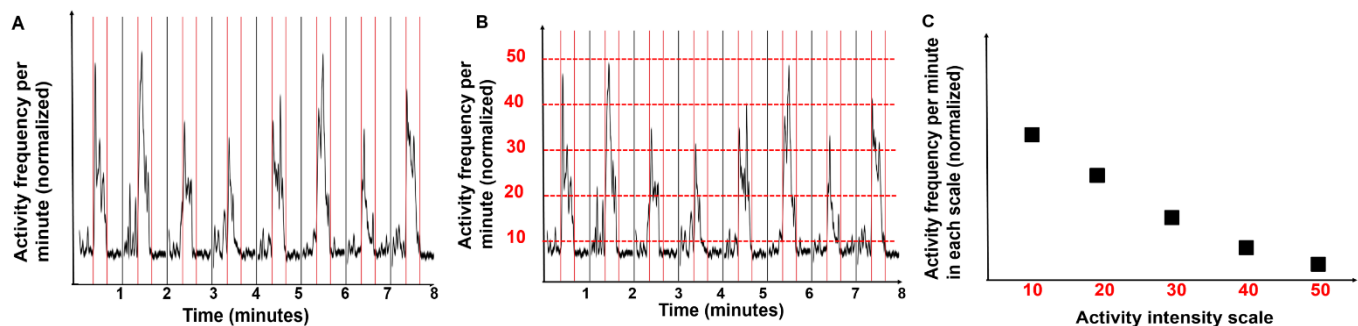


**Additional file 1: Figure S4. *shank3b* target-mutation in zebrafish via CRISPR-Cas9 system. (A)** Sanger sequencing result of WT and *shank3b* site mutations in zebrafish. *shank3b*<sup>-/-</sup> zebrafish has a 5-base deletion and a 13-base insertion. **(B)** *shank3b* protein of WT zebrafish and *shank3b*<sup>-/-</sup> predicted translated protein sequences are shown. Protein spatial structure prediction results of WT.





**Additional file 1: Figure S5. Examinations of maternal or paternal origin effects on the morphological and behavioral phenotypes. (A)** Morphological examination of the *shank3b*<sup>-/-</sup> ♀ and *shank3b*<sup>-/-</sup> ♂ offspring at 1 dpf. **(B-D)** Activity of *shank3b*<sup>-/-</sup> ♀ and *shank3b*<sup>-/-</sup> ♂ offspring at 2 dpf, 5 dpf and 7 dpf. **(E-F)** Light/dark response of *shank3b*<sup>-/-</sup> ♀ and *shank3b*<sup>-/-</sup> ♂ offspring at 5 dpf and 7 dpf. *shank3b*<sup>+/-</sup> ♀ were obtained from crosses of *shank3b*<sup>-/-</sup> ♀ with WT ♂ zebrafish. *shank3b*<sup>+/-</sup> ♂ were obtained from crosses of *shank3b*<sup>-/-</sup> ♂ with WT ♀ zebrafish. There were no significant differences between the two groups in **(B'-D')** activity test and **(E'-F')** light/dark response test (except in the second light response). N=24 for each group.



**Additional file 1: Figure S6. Analysis of activity frequency at different activity intensity scale.**

Viewpoint's zebrafish was used to track the larvae and ViewPoint's zebrafish system was used to analyze the larvae activity, as reported before [1, 2]. **(A)** The X-axis shows the time, and the Y-axis shows the activity frequency traveled by larvae in each 1-minute time bin. **(B)** To further analyze the variances of different activity intensity scales among WT, *shank3b*<sup>+/-</sup> and *shank3b*<sup>-/-</sup> zebrafish, we divided the activity equally into five levels (10, 20, 30, 40 and 50). **(C)** Next, we calculated the activity frequency of different activity intensity scales.

[1]. Rihel, J., et al., Zebrafish Behavioral Profiling Links Drugs to Biological Targets and Rest/Wake Regulation. Science, 2010.

[2]. Kokel, D., et al., Rapid behavior-based identification of neuroactive small molecules in the zebrafish. Nature Chemical Biology, 2010.